Proceedings of the Iowa Academy of Science

Volume 70 | Annual Issue Article 29

1963

The Demonstration of Mitotic Figures in Green Algae

William R. Bowen
State University of Iowa

portion of the normal nucleolar organizer to another chromosome, as has been observed in maize (5, 6), seems remote at this time. Since the normal nucleolus occurred in all cells along with the nucleolar-like body, any hypothesis of a deficiency of the normal nucleolar organizer seems equally unlikely. The two plants containing the organelle have been crossed together and to normal plants, but the progeny from these crosses have not been examined at this time. From the results of these crosses the mode of inheritance of the organelle will be determined.

Literature Cited

1. Duncan, R. E., and J. G. Ross, 1950. Jour. Heredity 41:259-268.
2. Burnham, C. R., F. H. White, and R. Livers. 1954. Cytologia 19:191-202.
3. Bemis, W. P. and G. B. Wilson. 1953. Jour. Heredity 44:91-95.
4. Navashin, M. 1934. Cytologia 5:169-203.
5. McClintock, B. 1934. Z. Zellforsch. 21:294-328.
6. Lin, Mei. 1955. Chromosoma 7:340-370.

The Demonstration of Mitotic Figures in Green Algae

WILLIAM R. BOWEN[1]

Abstract: Wittman's aceto-iron-haematoxylin stain was combined with Hoyer's mounting medium in a rapid method for demonstrating mitosis in the green algae. Both filamentous and unicellular forms were killed, fixed, and stained in one step with the self-mordanting stain. Cells were then washed with acetic acid and transferred directly to the water-soluble Hoyer's medium. Slides prepared in this manner are permanent. Chlorophyll was bleached from the algal cells by this medium. This revealed the stained nuclei and mitotic figures. This simplified technique is particularly suitable for student use.

The study of mitosis in the green algae is relatively difficult as compared to similar studies with the higher plants. Numerous cytological methods, e.g., aceto-carmine (1) and propionocarmine (2), have been employed successfully for chromosome counts but they are not very practical for student use. They involve the preliminary steps of killing and fixing the cells, followed by the bleaching of chlorophyll from the cells before staining. Plasmolysis and collapse of cells is frequently encountered when it is attempted to make these preparations permanent. A simple and practical cytological technique which eliminates these problems is described.

PROCEDURE

The combination of Wittman's aceto-iron-haematoxylin stain

[1]Department of Botany, State University of Iowa, Iowa City, Iowa

(3) and Hoyer's mounting medium (4) forms the basis for this technique. The stain is easily prepared by dissolving 2 gm of haematoxylin (National Aniline, cert. No. NH 15) in 50 ml of 45% acetic acid and then adding 0.5 gm to iron alum (ferric ammonium sulfate). The stain, prepared at room temperature, should not be used during the first 24 hours. The stock solution keeps from 4 to 6 months when refrigerated. Hoyer's mounting medium is made by dissolving 30 gm of gum arabic (lump or flake) in 50 ml of distilled water, then dissolving 200 gm of chloral hydrate in this and adding 20 ml of glycerin (5). The medium should be kept in air-tight containers. If it does become too thick and concentrated, water may be added to thin it to the desired consistency.

Mitotic figures of both filamentous and unicellular species of green algae were successfully demonstrated with this technique. The following schedule has proven satisfactory for the filamentous form, *Oedogonium cardiacum*. Fresh filaments grown in soil-water culture (6) are placed directly in a drop of stain on a slide. (A peak of mitotic activity occurs from 2 to 5 A.M.; fortunately, mitotic figures are often obtainable from 5 to 7 A.M.). The stain, with no heating, is effective within 20 minutes. It is then drained from the slide and the filaments are washed with 45% acetic acid until all traces of stain precipitate are removed. The filaments, with a minimum of acetic acid, are transferred directly to a drop of Hoyer's medium placed on the slide. It is usually advantageous to spread the filaments with needles before applying a cover slip. If necessary, a weight on the cover slip overnight will aid in flattening both the algal cells and the medium.

The preceding schedule was slightly modified for the unicellular form, *Eremosphaera viridis*. This large, non-motile alga, easily grown on Waris medium (6), is best stained without removing the cells from the agar. A small piece of agar containing a clump of cells is placed directly in a drop of stain. The stain, effective within 2 to 3 minutes, is again removed with 45% acetic acid. The cells are now easily separated from the agar, using needles under a dissecting microscope, and are passed directly into Hoyer's. Mitotic figures in the cells of *E. viridis* are usually present from 12 to 3 A.M., although mitotic activity in this alga seems to vary more than it does in *O. cardiacum*.

Discussion

The acetic acid in Wittman's aceto-iron-haematoxylin stain functions as a cytological killing and fixing agent. Some "bubbling" of the cytoplasm may occur but no serious disruption of the mitotic figures results. Previous aceto-haematoxylin stains have been noted for their rapid fading (1), but this is not true of

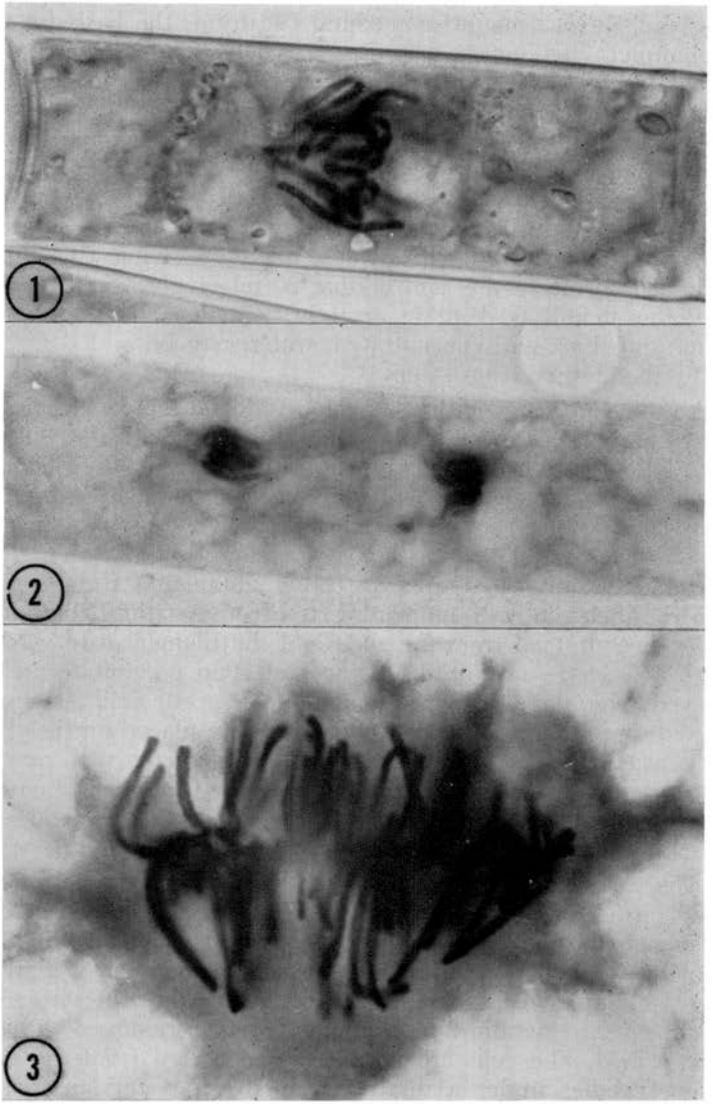

Figure 1. Metaphase of mitosis in *Oedogonium cardiacum* (X 1180)
Figure 2. Late anaphase of mitosis in *Oedogonium cardiacum* (X 1180)
Figure 3. Metaphase of mitosis in *Eremosphaera viridis* (X 1320)

Wittman's formula, in which the iron alum functions as a self-mordant. Preparations more than a year old retain their intensely stained chromosomes.

The time required for proper staining of the chromosomes in the green algae may vary from alga to alga but it appears that the filamentous forms usually require a longer period of time than do the unicellular ones. However, the chromosomes are

stained an intense purple-black within a relatively short time as is illustrated in the accompanying photomicrographs (Figures 1 to 3).

Utilization of Hoyer's mounting medium has been reported occasionally in the literature. Anderson (7) described the mounting of bryological materials in it and noted its effectiveness as a clearing agent. Beeks (4) used Hoyer's for permanent squashes of microsporocytes. The later noted that this medium was being used for preparing chromosome mounts of several species of green algae, but apparently this technique was not published.

The advantages of Hoyer's as a cytological mounting medium are numerous. It is permanent and rarely undercuts. If too much acetic acid is transferred into the medium along with the algal cells, undercutting and evaporation do result. When the slides do not contain the desired cytological material, the slides and cover slips are easily reclaimed by soaking in water. Morevoer, Hoyer's is a water-soluble medium with a low viscosity which permits algal cells to be transferred directly to it with no intermediate steps of dehydration, etc., involved. Hoyer's also eliminates the need for preliminary bleaching of the chlorophyll which partially or completely masks the stained nuclei and mitotic figures in the cells. Bleaching is normally complete when the preparations are left overnight, but enough bleaching does take place within 15 to 30 minutes to permit observation for mitotic figures.

The protoplasts of algal cells are quite viscous and do not lend themselves to chromosome counts. Attempts to prepare squashes without previous chemical treatment only disrupt cell organization and the mitotic apparatus. Venkataraman and Natarajan (2) used a prefixation treatment of either colchicine or 8-hydroxyquinoline to contract the chromosomes of *Rhizoclonium* while reducing the viscosity of the protoplasts. Such pretreatments have not been utilized here since this technique is primarily one for the whole mount demonstration of the mitotic apparatus.

The simplicity of this cytological technique makes it a useful and practical tool for the student in high school or college. It is easily adapted to either filamentous or unicellular species of green algae. Besides illustrating the mitotic apparatus, it may be used, for example, to demonstrate quickly the binucleate condition of certain species of *Rhizoclonium* and the multinucleate cells of *Cladophora* or *Pithophora*.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Drs. H. L. Dean and R. L. Hulbary for their encouragement and criticism.

Literature Cited

1. Godward, M. B. E. 1948. Nature (Lond) 161:203.
2. Venkataraman, G. S., and K. V. Natarajan. 1959. Stain Technol. 34: 233-234.
3. Wittman, W. 1962. Stain Technol. 37:27-30.
4. Beeks, R. M. 1955. El Aliso 3:131-134.
5. Alexopoulos, C. J., and E. S. Beneke. 1962. Laboratory Manual for Introductory Mycology. Burgess, Minneapolis.
6. Starr, R. C. 1960. Am. J. Botany 47:67-86.
7. Anderson, L. E. 1954. Bryologist 57:242-244.

A Preliminary Study of Chromosome Number in Oak Sprouts[1]

DAVID F. CONOVER[2]

Abstract. A preliminary study of chromosome numbers in oak sprouts was undertaken to determine the frequency of polyploid stump sprouts. A satisfactory method of making slide mounts using lateral bud primordia of newly burst buds was developed. None of the limited number examined to date was polyploid.

For some time it has been known that sprouts developing on excised stems are occasionally polyploid. Jorgensen (1928) was able to obtain up to 10 per cent tetraploid tomatoes by decapitating young tomato plants and removing all buds. A callus tissue developed on the wound surface producing numerous shoots, most of which were diploid. Lindstrom and Koos (1931) using homozygous diploids (from a sprouting haploid) obtained about 32 per cent tetraploid sprouts. The possibility that some of the peculiarities observed in oak stump sprouts might be attributed to polyploidy was the incentive for a study of chromosome numbers in oak stump sprouts.

MATERIALS AND METHODS

A preliminary study, primarily to work out a technique of determining chromosome number of sprouts, was initiated in late December 1961. Twenty-six pin oak (*Quercus palustris* Muenchh.) sprouts were collected and placed in the greenhouse on December 27. All 26 were taken from two small 4 in. diameter stumps in the State Conservation Commission nursery south of Ames. Additional sprouts were collected March 12, 1962. Each sprout was placed either in tap water or in moist sand within a plastic terrarium. As soon as the buds began to break dormancy and had elongated ½-1 in., collections were made. Buds were placed

[1] Journal Paper No. J-4335 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1047. In cooperation with the United States Department of Agriculture. Sincere appreciation is due Dr. H. S. McNabb, Jr. for encouragement during the course of this study.
[2] Department of Biology, Wisconsin State College, Oshkosh, Wisconsin.